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Author Affiliation:

Research scholar, Dept. of Geology, Institute of Biobidzhan, Russia

Correspondence to:

Research scholar, Institute of Biobidzhan, Russia, Mail: zhantmich@yandex.ru, Mobile No: (+7) 9241501316

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Genotoxicity effect of polluted water collected from Adaiyar region of river Cooum on Karyology of inhabiting fish, *Oreochromis mossambicus* (Peters)

Krishnamurthy R ✉, Elumalai K

ABSTRACT

In the present investigation was aimed to study the impact of polluted water collected from the river Cooum, at Adaiyar, Chennai, Tamilnadu on the karyomorphology of chromosomes in the fish, *Oreochromis mossambicus* inhabiting the study area. The experimental fish fingerlings were collected from the site and acclimatized in the laboratory. Then the fishes were exposed to different exposure periods and the impact of pollutants from the water was analyzed for its genotoxic effects on *O. mossambicus* chromosomes. The results of the present investigation clearly revealed that the river water pollutants induced several chromosomal abnormalities such as endoreduplication, addition and deletion of chromosomal arm, fragmentation, Nucleolar Organizer Region, Ring chromosomes, condensation of chromosomes with dark staining, indistinct chromosomal contour etc. Thus this present study focuses more attention on the Genotoxicity of pollutants on non-target organisms like fish.

Key Words: Pollutants, chromosomal aberrations, NOR, *Oreochromis mossambicus*.

1. INTRODUCTION

Mother nature has provided immense natural resources for human beings. Water is one such natural resource which serves man's basic needs without which not only man but no life can exist on earth. Intervention, in the form of release of pollutants by man into the environment in terms of addition or depletion of substance or energy is liable to cause hazards to human health, have to other, living resources and damage to the harmoniously balanced ecosystem.

Besides, these interventions would indirectly interfere with the legitimate use of the environment. Moreover, population chronically exposed to these pollutants often respond by exhibiting variation in their genomic expression, learning to changes in phenotype, psychology and behaviour which culminate in the evolutionary plasticity. However, most studies fail to clearly define the basis for such population response to contaminants. Periodic exposure of small water bodies to extreme drought condition and failure of rains with high atmospheric

temperature causing rapid evaporation can easily turn the left over water column derelict and hostile to the aquatic fauna, particularly fish species.

Fish have survived millions of years in the most diverse, adverse and advanced environments. It is expected that all known mechanisms of chromosomal changes could have occurred in the evolution resulting in a characteristic karyotype. Mercury released through effluents is accumulated in organic form in fish and other marine organisms. Local people who consume fish from the polluted water have to face grave consequences. The rivers Coovum and Adyar pass through the city of Madras and finally reach the Bay of Bengal near Chepauk and Foreshore estate respectively (Plate 1). River Coovum (12°5' N, 70° 35'E to 13° 1'N, 80° 15E) winding its course across the heart of the city, until its joining the Bay of Bengal has been considered to be an endowment to the city. The Coovum basin has a catchment area of 86.4 sq.km of which about 18 sq.km lie within the city limits (Sunderesan, 1957). River Coovum starts as a rain course collecting the surplus of about 75 small tanks to the minor basis starting Tiruvalluvar taluk of Chinglepet district of Tamil Nadu. It flows through 65 km and it is linked up through man made channel with the river Korataliyar flowing just north of the city. There is a small reservoir at Korattur from where the New Bangaru channel limits to Chembarapakkam tank. The Coovum River flows in the North East direction upto Thiruvallur railway station and then turns east. It flows a course of 12 km adjacent to Tiruvallur - Thirumazhisai - Poonthamallee Road.

Coovum enters the city near Arumbakkam and flows through Choolaimedu, Chepet, Egmore and Chintadaripet. Near Central railway station it branches off into two arms and forms an island. The northern arm flowing around island ground contains the waters of both the Coovum and Buckingham canal. The Buckingham canal leaves the Coovum near the campus of University of Madras at Chepauk, and the Coovum passing under the Napiar Bridge, enters the Bay of Bengal. The river bed is 40' wide from Arumbakkam to co-optex point of Egmore from there to Periyar Bridge the width is 120' thereafter the width is 300' upto the Napiar bridge. The entire stretch of 11 km is having an area of 0.325 sq.km. The rate of evaporation during summer may be 18.5 lakhs l/day. The total sewage inflow is around 15 lakhs l/day (Joseph et al., 1992).

Main source of pollution in Coovum is the Madras Metropolitan Water Supply and Sewage Board (MMWSSB) which let off about 50 million gallons of sewage water into the Coovum at the site of Arumbakkam. Coovum carries sludge, domestic sewage, cattlewash, effluent discharges from industrial establishments of Ambattur and Padi complex and also government and private sectors. In its physical aspect, the river Coovum produces three types of odours: normally the smell of faeces and hydrogen sulphide is common; however, during the monsoon, there is a distinct ammonia smell; and in summer due to the algal blooming in the river, camphor like smell emanates from it.

During the rainy season the gaseous pollutants are washed down as acids adding to the pollution of land and water. Solid wastes like salt dust, if stored, are likely to be washed away during rain causing pollution of ground water and finally the river Coovum. During dry weather the flow of river Coovum consists almost entirely of effluents. It must be noted that most of the pollutants traveling down the river will eventually end up in the sea. Domestic sewage from rapidly expanding suburbs contains in addition to oxidizable materials, detergents, nutrients, heavy metals, pathogens as well as waste compounds of unknown lethality/sub lethality all of which are directed untreated into the nearest Coovum. Industrial effluents consist of a variety of substances of either known or unknown lethality. Most water sources will receive a number of industrial effluents either directly or indirectly.

Karyological information of fish is becoming increasingly used in the field of evaluation, cyto-taxonomy, gene mapping, mutagenesis and aquaculture. The research on fish chromosome is scanty when compared to other animal groups, because of the lack of reproducible techniques for obtaining high quality metaphase spread.

Currently studies on the chromosomes are gaining more importance due to their application in hybridization which can contribute to the improvement of fish culture. The efficiency of chromosomal engineering programmes in producing haploid, parthenogenetic, and polyploid organisms can be most accurately assessed by karyotyping and hence this attempt of analyzing the environmental observation confused by the polluted water on the fresh water fish, *Oreochromis mossambicus* (Peters) inhabiting in the river Coovum was taken in the present study.

2. MATERIALS AND METHODS

Water samples were collected from the Adaiyar region of the river Coovum. Water samples (10 litres) were collected in polythene containers and immediately taken to the laboratory and analysed for their physicochemical parameters were analyzed following the standard methods of American Public Health Association (APHA, 1989).

2.1. Experimental fish, *Oreochromis mossambicus*

Selection of experimental fish was made on the basis of economic importance, availability and suitability to experimental purpose. For the present investigation *Oreochromis mossambicus* was collected from the study area at river Coovum and acclimatized in the laboratory.

2.2. Karyological Protocol

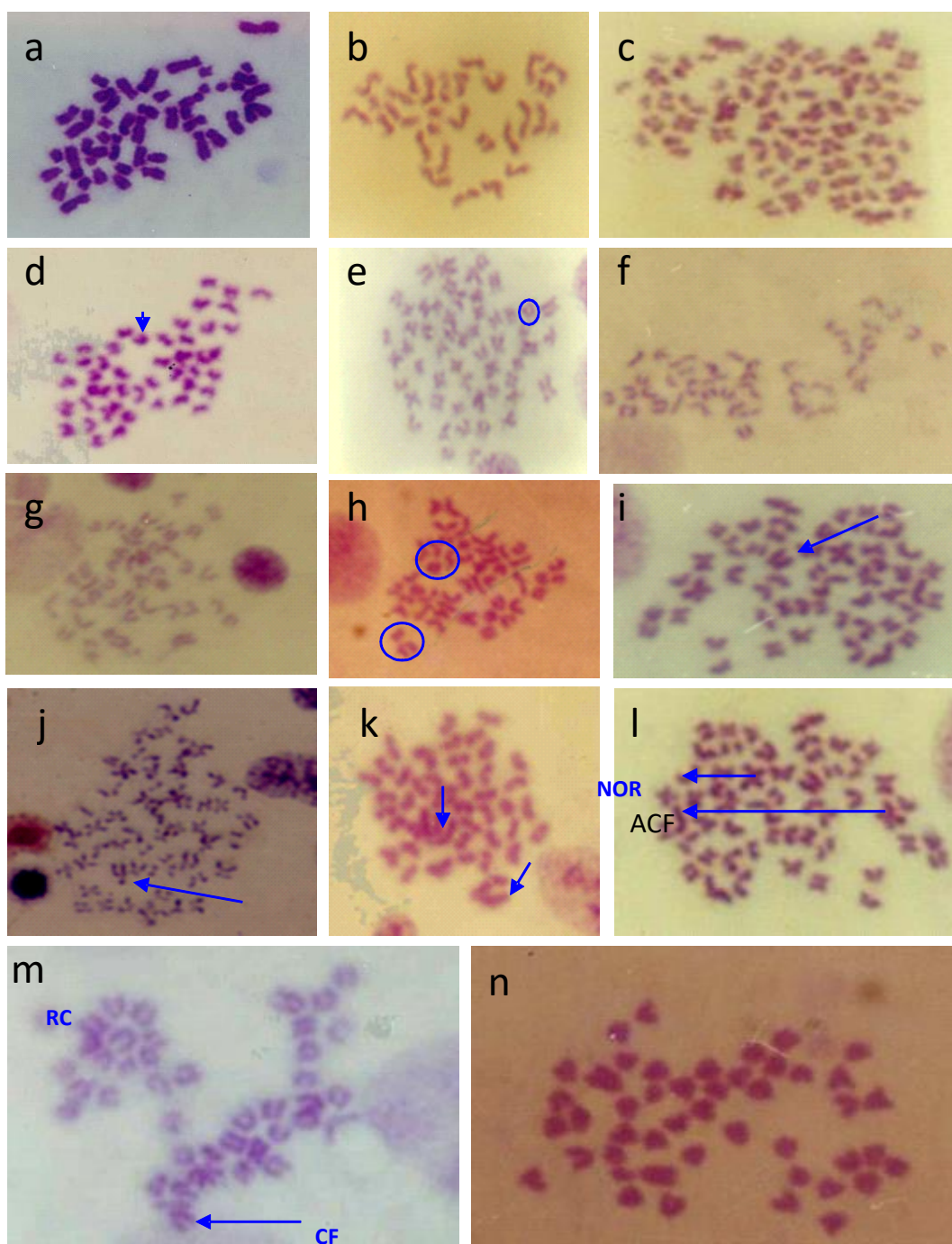
Several methods have been employed in for karyology and the most successful of these have been tissue culture-colchicine rapid chromosome preparation from solid tissues (Kligerman & Bloom, 1977), injection and squashing of the testis or haemopoietic tissues (Roberts, 1964; and Ohno *et al.*, 1965) and squash from embryological material. All of these methods have given usable results, but are time consuming and yield low percentage of countable chromosomes. The following technique yields a high proportion of countable chromosome sets from a single preparation in a very short time, and has the virtue of leaving the specimen intact for further taxonomic studies. At present the technique is empirical and may have to be modified for some groups of fishes. It is most successful on fresh water fishes and has given usable results, in several marine groups also. The procedure given below is for juvenile fishes of 2-6 inches in standard length. For larger fishes both the colchicine dose and the length of time after injection should be increased.

An intramuscular injection of 0.1 ml of 0.01-0.05 % (depending on the size of fish) of colchicine was administered in the anterior portion of the back. The injected specimens were released in to the container with natural water for 2 - 3 hrs. After 2-3 hrs, the fish was dissected out for isolating gills, kidney. Eventually the gill, kidney, were transferred to an embryo cup containing 2 ml of 0.4 % of KCl solution for 30 minutes. Tissues were centrifuged at 800 rpm for 10 to 15 minutes and the supernatant removed leaving cell button at the bottom. Centrifuge tubes with sample were kept in room temperature for 20 – 30 minutes. Again the samples were centrifuged at 800 rpm for 5 minutes and the supernatant removed leaving the cell button. Subsequently the samples were fixed in a solution thrice at an interval of 20-30 minutes in each; the fixation was made in a solution of 3: 1 ratio of methanol: acetic acid (Carnoy's fluid).

Supernatant fixatives were removed leaving the suspension at the bottom. The fixed samples were kept at low temperature in a refrigerator. Pieces of tissues were removed from the fixed material and blotted on a filter paper to remove the fixative and placed in the well of cavity slide containing 2-5 drops of 50% acetic acid. The tissues was minced with sample or fine dissecting needle for a minute to make a cell suspension (Remaining tissue fragments were replaced in fixatives). Using fine pasture pipette or microhaematocrit capillary tube with a rubber bulb, a drop of the above suspension was drawn and expelled into the acid clean slide and warmed at 40-50°C. Quickly a drop was sucked back into the pipette to leave a ring of cells and it was repeated a few times to have a number of concentric rings. The above procedures (i & j) were repeated to produce 2 sets of rings on each slide. The preparation was stained in 4% Giemsa stain at pH 6.8 for 20 minutes. Excess stain was removed by washing in distilled water. The metaphase spreads were easily located using the Carl Zeiss photomicroscope III in the periphery of the rings. The same procedure was adopted for both control and the experimental fish.

3. RESULTS

As per the methodology, the gill tissue were prepared for chromosomal spread and viewed through photomicrograph. About 15 specimens were used for this purpose. From both the sites to assess the number of chromosomes (2n) and also the chromosomal variations (aberrations) if any. The chromosomal complement in *O. mossambicus* was found to be 44 (2n=44) in the control fish. Nevertheless, a few diploid metaphase complement shows variations. It can be attributed to intricacies involved in the methodologies and variation in the uncertainty of the chromosomal structure. All the chromosomes showed distinct morphology with a clear position of centromeres. Significant variation have been found between the control fish collected from the experimental sites. Environmental and chromosomal variation have been two sides of a coin as they are inseparable. Off late the impact of environmental factors on the biosystem results in genomic aberrations in the form of ploidy. In the same way Endoreduplication is an internal doubling of chromosomes resulting from two successive DNA synthesis without intervening cytokinesis. Sister chromosomes are paired to form diplochromosomes and they show three phases of changes. I phase in twine SCE, II phase of single SCE and III phase of intra diploid chromatid interchange which occurs between chromosomes in diploid chromosomes.



PLATES : a=Normal metaphase chromosome of *O. mossambicus*; b=metaphase plate showing decreased number of chromosomes; c=Endoreduplication; d= Metaphase plate showing condensed morphology Note: Arrow Indicates fragment of chromosome; e= Poorly stained chromosomes showing ring chromosomes - circle indicates ring chromosome; f= Indistinct morphology and rod chromosomes; g= Opaque chromosomes with poor staining property; h= ring chromosomes are encircled; i=Odd number of abnormal chromosome; j=Chromosome showing deleted arm; k=Three abnormal size of chromosomes with diad nature; l=NOR Nucleolar Organizer Region , ACF Addition of Chromosomal Fragment; m=RC (Ring Chromosome) & CF (Chromosomal Fragment); n= over stained chromosome with indistinct karyomorphology

3.1. Ploidy and endoreduplication in the experimental fish, *O. mossambicus*

There has been a vast difference between the control and the experimental samples. In their diploid configuration as its is evidenced from plate 2. The control gill cells shows no aberrations which the experimental gill cells show a phenomenal increase in their chromosomal number increase or decrease (Plate 2 B and C). It is worth to emphasize that the control sample reveals absolute zero percentage of endoreduplication., Further more metaphase plates obtained from the gill cells of *O. mossambicus* possess the chromosomes with condensed morphology (Plate 3A) along with that, a piece of chromosome showing odd size were also noted, their staining property was more than the control. A few metaphase plates showed tetrad chromosomes most of them are metacentric and submetacentric. One ring chromosome was also noted in their complement (Plate 3B). Chromosomal analysis from the same sample also showed chromosomes in dead condition (Plate 3C). Similarly, the fish collected from the site 2 showed opaque chromosomes which has been poorly stained Karyomorphology and their nature is really confusing because, in that complement, it was unable to found the upper arm of the chromosomes among them.

A pair of chromosomes got strong straining and appears to be bigger in that complement. It can be attributed that it may be the sex chromosomes because no other partner chromosome was found in the complement. Certain chromosomal aberrations like ring chromosome, red chromosomes and secondary constriction region was also noted. Only one chromosome with abnormal karyomorphology have been appeared in the karyomorphology of gill cells of the experimental fish *O. mossambicus* from the experimental site (Plate 1). It is interesting to note that the metaphase plate obtained from the *O. mossambicus* has lost is one arm (upper arm arrow indicates the division of arm from tetrad chromosomal anomaly. That is, chromosomal gap (a small space in one arm of the chromosome) have been observed in the fish collected from the same site. Nucleolar organizer Region (NOR) and Hetero-Chromatin Region (HCR) are common phenomenon observed in the metaphase complement of the experimental *O. mossambicus*. An addition of a treatment of chromosome was also noted in the same complement. Ring chromosome over stained chromosomes were also noted in the metaphase complement from the fish collected from Adaiyar site. It may be attributed due to over load of sediments, because the river Coovum act as a junction point which receives several side channels from many parts of the city.

4. DISCUSSION

The literatures concerned to the effect of pollutants on fish are scanty and deserves more attention. Chromosome number, form, size and points of spindle fibre attachment provide another interesting tool to help decipher the evolutionary relationship of fish. Although karyology is still in an early stage of development in fish, nevertheless, promising for unraveling the interrelationship among the various genera of fish. The test organism, *O. mossambicus* showed different types of chromosomal variations in the present analysis may be attributed to the inducement of heavy metals present in the river Coovum and this observation is no commitment with Millar (1972). Prior to this study, the toxic effects of Cd had been studied in the rainbow trout *Oncorhynchus mykiss* for its role in carcinogenesis (Metcalf, 1989), as well as for toxicopathological symptoms and bioaccumulation (Iliopoulou-Georgudaki and Kotsanis, 2001; Dang et al., 2001). However, to our knowledge, genotoxicity induced by aquatic pollutants alone or in combination with heavy metals, had not been documented before, in this or any other fish model (Kar and Das, 1987; IARC, 1993; Mavournin et al., 1990). In the present study, the results indicated that there was a greater extent in increase of chromosomal variations noticeable between the two sites of river Coovum, the Saidapet site showing a greater number of chromosomal anomalies. Additionally, along with the elevated frequencies of ploidy and endoreduplication, there was an altered chromosomal contour observed in gill tissues of *O. mossambicus* as a result of heavy metal present in the water vis-à-vis control.

As reported by earlier workers, the data on change in protein features noted during our study have not been included for their possible future and further study of interest in the field of toxicology (Voet and Voet, 1995). Presently, the change in protein content or profile induced by a mutagen might also be considered as a potential indicator of genotoxicity, as suggested in earlier studies as well (Guha and Khuda-Bukhsh (2002a); Guha and Khuda-Bukhsh (2002b) and Guha, Khuda-Bukhsh (2003) and Weigant et al., (1997). The present observations on protein may represent a first step in the process of determining whether or not the expression of the DNA, in the form of protein products, has been affected by the chemicals used, because expression of certain classes of proteins (e.g., metallothionein, stress proteins) are known to be affected in very specific ways upon exposure to Cd and other chemicals that produce genotoxic stress in an organism (Dang et al., 2001; Hartwig, 1994; Tsuzuki et al., 1994; Klopman et al., 1985; Awasthy et al., 1995; Sies, 1993; Rosenkranz and Klopman, 1995). Therefore, the calculated electronegativity of Aza is of the same order of magnitude as that for DNA-reactive molecules, showing thereby all the features of a potential mutagen capable of inducing damage in genetic material, including the clastogenic changes observed in the fish under study. However, it is not clearly understood why and how Aza tended to ameliorate the effects of CdCl₂-induced clastogenic changes. One hypothesis to explain

such modulation could be that Aza competed in some way to block the DNA-binding sites on which CdCl₂ had disruptive effects on binding.

5. CONCLUSIONS

The results of the present study suggest that the natural water resources such as rivers, pools, streams and lakes should be protected from pollution. Common awareness programme oriented towards the impact of polluting the environment by simply discharging them into the system will have deleterious effects over the non-target organisms like fish and man. This in turn affects the future, by the accumulation of mutagens in parents who are consuming the fish from polluted water will give rise to teratoma. Hence, further studies on this aspect are urgently needed to find out the impacts of these pollutants on the health aspects of human beings.

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Conflicting interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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